works but it is thought that an initial hydrolysis of PtdIns(4,5)P₂ to inositol trisphosphate and diacylglycerol takes place, but an accompanying hydrolysis of PtdIns cannot be ruled out. Inositol trisphosphate and diacylglycerol may be 'second messengers', involved in intracellular calcium mobilization and protein phosphorylation respectively (Berridge, 1984). As a result of the phosphoinositide effect there may also be an increase in permeability of the plasma membrane to calcium, which has been linked to short-term memory (Penniston, 1983).

Samples of normal and Alzheimer-diseased human brain were obtained from the M.R.C. Brain Tissue Bank, Addenbrooke's Hospital, Cambridge, U.K. Bodies had been placed at 4°C within 4h of death and brains were stored at −70°C. For inositol analysis, 0.1 g of brain was weighed on a torsion balance and homogenized at 4°C in 2.6 ml of deionized water by using a glass Potter homogenizer with motorized Teflon Pestle. Protein was removed by precipitation with cold 2.5 M ammonium acetate acetone-water 80:20.6:1.64 vol., and the mixture was centrifuged at 100000 × g at 4°C for 1h. After centrifugation, samples were lyophilized and phosphorus was determined by a modification of the method of Bartlett (1959). Activation of muscarinic receptors in bovine adrenal medulla causes rapid polyphosphoinositide breakdown without calcium mobilization

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Activation of certain receptors causes polyphosphoinositide breakdown without accompanying influx of Ca²⁺ from the extracellular space, and it has been suggested that inositol 1,4,5-trisphosphate liberated by hydrolysis of PtdIns(4,5)P₂ acts as a second messenger to mobilize Ca²⁺ from internal stores, probably associated with endoplasmic reticulum (Berridge, 1984). Nicotinic receptor activation in bovine adrenal medulla causes Ca²⁺ influx and catecholamine secretion, though no changes in phosphoinositide metabolism are observed (Fisher et al., 1981). However, muscarinic receptor stimulation causes inositol lipid changes in these medullary chromaffin cells, simultaneously depressing the secretion due to nicotinic drugs (Swilem et al., 1983) but causing no Ca²⁺ influx (Fisher et al., 1981). This indicates that the phosphoinositide response and Ca²⁺ influx are separate events, each modulated by a different receptor type. It is thus difficult to accept theories linking Ca²⁺
influx with phosphoinositide metabolism in the adrenal medulla (Hawthorne, 1982).

Using chromaffin cells in culture, we have explored the identity of the first inositol lipid hydrolysed in response to muscarinic stimulation, the dependence of this hydrolysis on external Ca$^{2+}$ and the effect of muscarinic activation on intracellular Ca$^{2+}$.

Cell isolation was carried out essentially as described by Knight & Baker (1983). For cell culture the method of Fisher et al. (1981) was used. The cultured cells were prelabelled for 75 min in 1 ml of Locke's solution containing 50 μCi of [32P]orthophosphate/ml and then washed twice with fresh Locke's solution. The cells were then replaced with Locke's solution containing cholinergic drug (or control Locke's solution only) and the brief incubation at 25°C terminated by the addition of 1 ml of 20% trichloroacetic acid and approx. 20 mg wet weight of adrenal medulla homogenate, this latter as 'carrier' tissue. The precipitate was centrifuged down, washed once with 1 ml of 5% trichloroacetic acid containing 1 mM-EDTA and once with 2 ml of distilled water.

Lipid was extracted from the washed pellets with chloroform/methanol/conc. HCl (100:100:1, by vol.), a two-phase system then being produced by the addition of chloroform and 0.1 M-HCl. The lower phase was concentrated under nitrogen for application to thin-layer plates of silica gel 60H spread in 3% magnesium acetate. Two-dimensional separations employed chloroform/methanol/ammonia (65:25:5; by vol.) followed by chloroform/acetone/methanol/acetic acid/water (50:40:10:10:5; by vol.). After iodine staining spots were scraped off and radioactivity determined by liquid scintillation counting.

Intracellular Ca$^{2+}$ was monitored by using the fluorescent indicator Quin 2 (Tsien et al., 1982). Cells were prepared for incubation by washing with fresh 20 mM-Quin 2-AM and washed twice in Ca$^{2+}$-free Locke's solution. The cells were then transferred to the reaction cuvette, to which CaCl$_2$ (1 mM) was added followed by the cholinergic drug.

Table 1 shows that incubation of prelabelled chromaffin cells with carbachol causes a loss of PtdIns(4,5)P$_2$ and PtdIns4P within 30s. There is a concomitant increase in PtdIns4P within 30s. There is a concomitant increase in Ca$^{2+}$-labelling, possibly because of diacylglycerol kinase acting upon the diacylglycerol released from the phosphoinositides. The loss of polyphosphoinositides does not occur in Ca$^{2+}$-free medium, a result similar to those of Rhodes et al. (1983), using vasopressin-stimulated hepatocytes. However, the calcium-dependence is controversial in this system (Hawthorne, 1983). Methacholine causes a similar response to carbachol in the chromaffin cells, confirming that the phosphoinositide response is muscarinic.

These results cast doubt on the 'second-messenger' role of inositol trisphosphate in mobilizing Ca$^{2+}$ (reviewed by Berridge, 1984) since essentially the same PtdIns(4,5)P$_2$ loss is seen as in hepatocytes, but without Ca$^{2+}$ mobilization. In addition, rapid loss of labelled PtdInsP is also seen in response to muscarinic drugs, yet the resulting inositol bisphosphate is said to have no calcium-mobilizing properties (Streb et al., 1983).

The molecular events required for visual excitation are not completely understood. Any proposal describing this mechanism must account for signal transmission from the rhodopsin-containing discs to the plasmalemma, between which there is no observable continuity, and for the large-scale amplification which is thought to occur in the ROS (Hagins et al., 1970). Two major theories have been proposed to explain the transduction mechanism, one involving calcium as the primary transducer (Hagins, 1972), the other implicating cyclic GMP (Yee & Liebman, 1978; Fung & Stryer, 1980).

ROS contain a light-sensitive cyclic GMP phosphodiesterase. An analogy has been made between the activation of this enzyme, and the receptor-mediated activation of adenylate cyclase in various tissues (Abood et al., 1982). The second major class of receptors involved in cell activation is invariably associated with changes in cyclic GMP levels, together with raised intracellular calcium (Berridge, 1981). An analogy between calcium mobilization in ROS could also be made. Recent evidence implies that polyphosphoinositides play an important role in mediating the effect of this type of receptor (Hawthorne, 1983; Berridge, 1984). Other investigators

<table>
<thead>
<tr>
<th>Ca$^{2+}$ (mM)</th>
<th>PtdIns(4,5)P$_2$</th>
<th>PtdIns4P</th>
<th>Phosphatidate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.2</td>
<td>602 ± 130</td>
<td>332 ± 60</td>
</tr>
<tr>
<td>Carbachol</td>
<td>2.2</td>
<td>481 ± 101*</td>
<td>231 ± 81</td>
</tr>
<tr>
<td>Carbachol</td>
<td>0</td>
<td>580 ± 85</td>
<td>341 ± 75</td>
</tr>
<tr>
<td>Carbachol</td>
<td>0.5 mM-EGTA</td>
<td>584 ± 46</td>
<td>320 ± 105</td>
</tr>
</tbody>
</table>

**P < 0.05; ***P < 0.001. 

Polyphosphoinositide metabolism in response to light stimulation of retinal rod outer segments

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The molecular events required for visual excitation are not completely understood. Any proposal describing this mechanism must account for signal transmission from the rhodopsin-containing discs to the plasmalemma, between which there is no observable continuity, and for the large-scale amplification which is thought to occur in the ROS (Hagins et al., 1970). Two major theories have been

Abbreviations used: ROS, rod outer segments; PtdIns(4,5)P$_2$, phosphatidylinositol 4,5-bisphosphate; PtdIns4P, phosphatidyl-inositol 4-phosphate. 

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